

In the Specification

Please add the following paragraph at page 1, after the title:

RELATED APPLICATIONS

A1 This application is a divisional of U.S. Application No. 09/449,437, filed November 24, 1999, the entire teachings of which are incorporated herein by reference.

Please replace the paragraph at page 4, lines 6 through 16 with the following paragraph:

A2
FO/2280-5900-160
The invention also relates to an isolated cell that produces an antibody or antigen-binding fragment of the present invention, including those which bind to mammalian Bonzo and inhibit the binding of a ligand to the receptor. In one embodiment, the isolated cell is murine hybridoma 4A11 (also referred to as murine hybridoma LS212-4A11-30-8) deposited under ATCC Accession No. PTA-991. In another embodiment, the isolated cell is murine hybridoma 7A2 (also referred to as murine hybridoma LS212-7A2-32-1) deposited under ATCC Accession No. PTA-992. In another embodiment, the isolated cell is murine hybridoma 7F3 (also referred to as murine hybridoma LS212-7F3-8-7) deposited under ATCC Accession No. PTA-990. In another embodiment, the isolated cell is murine hybridoma 9G2 (also referred to as murine hybridoma LS212-9G2-7-2).

Please replace the paragraph at page 5, lines 9 through 18 with the following paragraph:

A3
The invention also relates to therapeutic methods in which agents which can bind to a mammalian Bonzo and modulate (inhibit or promote) a Bonzo function are administered to a subject in need of such therapy. In one embodiment, the therapeutic method is a method of treating a subject having an inflammatory disease. In another embodiment, the subject has a cancer or an infection (e.g., viral, bacterial, fungal). In another embodiment, the therapeutic method is a method of inhibiting a cellular response (e.g., Ca^{2+} flux, chemotaxis, exocytosis, respiratory burst). In another embodiment, the method is a method of modulating a Bonzo

A3 function. In another embodiment, SExCkine is locally administered to a subject to recruit Bonzo⁺ cells to the area of administration.

Please replace the paragraph at page 6, lines 1 through 4 with the following paragraph:

A4 The invention also relates to therapeutic methods in which targeting molecules are administered to a subject in need of such therapy. In one embodiment, the therapeutic method is a method of treating a subject having a tumor or a viral infection.

Please replace the paragraph at page 12, lines 3 through 9 with the following paragraph:

A5 Figures 26A-26F are fluorescence histograms showing that Bonzo expression is augmented by repeated activation of *in vitro* derived TR1 cells. Cells which had been stimulated by one round of activation (Figures 26A-26C) or two rounds of activation (Figures 26D-26F) were stained with anti-Bonzo mAb 7F3 (Figures 26B and 26E), anti-CCR4 mAb 1G1 (Figures 26A and 26D) or anti-CCR7 mAb 7H12 (Figures 26C and 26F). TR1 cells expressed increased amounts of Bonzo after repeated activation (compare Figures 26B and 26E).

Please replace the paragraph at page 19, lines 23 through 28 with the following paragraph:

A6 "Receptor-binding variants" of mammalian SExCkine proteins include receptor-binding fragments (e.g., proteolytic fragments), receptor-binding mutant proteins and receptor-binding fusion proteins which can be produced using suitable methods (e.g., mutagenesis (e.g., chemical mutagenesis, radiation mutagenesis), recombinant DNA techniques). A "receptor-binding variant" can be identified using a suitable receptor-binding assay such as a Bonzo-binding assay described herein.

Please replace the paragraph at page 32, lines 10 through 16 with the following paragraph:

A7
mAb 4A11 can be produced by murine hybridoma 4A11, also referred to as murine hybridoma LS212-4A11-30-8, which was deposited on November 24, 1999, on behalf of LeukoSite, Inc., 215 First Street, Cambridge, MA 02142, U.S.A. (now Millennium Pharmaceuticals, Inc., 75 Sidney Street, Cambridge, MA 02139, U.S.A.), at the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110, U.S.A., under Accession No. PTA-991. The invention relates to murine hybridoma 4A11, to the antibody it produces and to nucleic acids encoding the antibody.

Please replace the paragraph at page 32, lines 17 through 22 with the following paragraph:

A8
mAb 7A2 can be produced by murine hybridoma 7A2, also referred to as murine hybridoma LS212-7A2-32-1, which was deposited on November 24, 1999, on behalf of LeukoSite, Inc., 215 First Street, Cambridge, MA 02142, U.S.A. (now Millennium Pharmaceuticals, Inc., 75 Sidney Street, Cambridge, MA 02139, U.S.A.), at the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110, U.S.A., under Accession No. PTA-992. The invention relates to murine hybridoma 7A2, to the antibody it produces, and to nucleic acids encoding the antibody.

Please replace the paragraph at page 32, lines 23 through 28 with the following paragraph:

A9
mAb 7F3 can be produced by murine hybridoma 7F3, also referred to as murine hybridoma LS212-7F3-8-7, which was deposited on November 24, 1999, on behalf of LeukoSite, Inc., 215 First Street, Cambridge, MA 02142, U.S.A. (now Millennium Pharmaceuticals, Inc., 75 Sidney Street, Cambridge, MA 02139, U.S.A.), at the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110, U.S.A., under Accession No. PTA-990. The invention relates to murine hybridoma 7F3, to the antibody it produces, and to nucleic acids encoding the antibody.

Please replace the paragraph at page 33, line 28 through page 34, line 14 with the following paragraph:

A10
09/449,437-082701

The invention also relates to a bispecific antibody, or functional fragment thereof (e.g., F(ab')₂), which binds to a mammalian Bonzo and at least one other antigen (e.g., tumor antigen, viral antigen). In a particular embodiment, the bispecific antibody, or functional fragment thereof has the same or similar epitopic specificity as mAb 4A11, mAb 7A2, mAb 7F3 or mAb 9G2 and at least one other antibody. Bispecific antibodies can be secreted by triomas and hybrid hybridomas. Generally, triomas are formed by fusion of a hybridoma and a lymphocyte (e.g., antibody secreting B cell) and hybrid hybridomas are formed by fusion of two hybridomas. Each of the fused cells (i.e., hybridomas, lymphocytes) produces a monospecific antibody. However, triomas and hybrid hybridomas can produce an antibody containing antigen binding sites which recognize different antigens. The supernatants of triomas and hybrid hybridomas can be assayed for bispecific antibody using a suitable assay (e.g., ELISA), and bispecific antibodies can be purified using conventional methods. (see, e.g., U.S. Patent No. 5,959,084 (Ring *et al.*) U.S. Patent No. 5,141,736 (Iwasa *et al.*), U.S. Patent Nos. 4,444,878, 5,292,668, 5,523,210 (all to Paulus *et al.*) and U.S. Patent No. 5,496,549 (Yamazaki *et al.*)).

Please replace the paragraph at page 34, line 15 through page 35, line 8 with the following paragraph:

A11

In another embodiment, the antibody or antigen-binding fragment thereof has specificity for a mammalian SExCkine, preferably a naturally occurring or endogenous human SExCkine. Such antibodies and antigen-binding fragments can be produced by a variety of suitable methods, such as those described herein. In one embodiment, the anti-SExCkine antibody can be raised against an appropriate immunogen, such as an isolated soluble and/or recombinant SExCkine or portions thereof (including synthetic molecules, such as synthetic peptides). Antibodies can also be raised by immunizing a suitable animal (e.g., mouse) with cells which express the transmembrane form of SExCkine. In another embodiment, the antibody is an IgG or antigen-binding fragment of an IgG. In another embodiment, the antibody is a human antibody or an

antigen-binding fragment thereof. In another embodiment, the antibody is a humanized antibody or an antigen-binding fragment thereof. In a preferred embodiment, the antibody or antigen-binding fragment can bind to a mammalian SExCkine and inhibit (reduce or prevent) the binding of the chemokine to receptor (e.g., Bonzo), and thereby inhibit one or more functions mediated by receptor in response to SExCkine binding. For example, the anti-SExCkine antibody can inhibit SExCkine-induced chemotaxis of Bonzo⁺ cells. Other functions which can be mediated by SExCkine binding to receptor (e.g., Bonzo) include, for example, signal transduction (e.g., GDP/GTP exchange by receptor associated G proteins, transient increase in the concentration of cytosolic free calcium [Ca²⁺]_i) and receptor-mediated processes and cellular responses (e.g., proliferation, migration, chemotaxis, secretion, degranulation, inflammatory mediator release (such as release of bioactive lipids such as leukotrienes (e.g., leukotriene C₄)), respiratory burst).

Please replace the paragraph at page 38, lines 9 through 17 with the following paragraph:

The first binding moiety can be, for example, an antibody which binds mammalian Bonzo or antigen-binding fragment thereof (e.g., Fab, Fv, Fab', F(ab)'₂), a Bonzo ligand (e.g., mammalian SExCkine, mammalian platelet factor 4) or Bonzo-binding variant of a ligand. The second binding moiety can be, for example, an antibody or antigen-binding fragment thereof which binds to a molecule expressed on the target cell or antigen binding fragment thereof. Where the targeting molecule comprises a first binding moiety which is an anti-Bonzo antibody or antigen-binding fragment thereof, it is preferred that said anti-Bonzo antibody does not inhibit binding of ligand to Bonzo.

Please replace the paragraph at page 54, lines 15 through 17 with the following paragraph:

In another embodiment, the invention relates to a method of promoting Bonzo mediated homing of leukocytes in a subject, comprising administering an effective amount of a promoter (e.g., agonist) of Bonzo function.

Please replace the paragraph at page 57, lines 14 through 17 with the following paragraph:

A14

- immunosuppression, such as that in individuals with immunodeficiency syndromes such as AIDS, individuals undergoing radiation therapy, chemotherapy, or other therapy which causes immunosuppression; immunosuppression due to congenital deficiency in receptor function or other causes.

Please replace the paragraph at page 66, lines 17 through 27 with the following paragraph:

A15

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Human multiple tissue northern blots I and II and a cancer cell line blot (Clontech) were used to analyze expression of the gene encoding the Bonzo ligand. cDNA probes were labeled with $\alpha^{32}\text{P}$ -dCTP by priming with random hexamers. A 400 bp fragment representing most of the chemokine domain of SExCkine cDNA cloned in pCDEF3 (from the 5' EcoR1 site (within vector pCDEF3) to an EcoRV site of a cDNA encoding human SExCkine (SEQ ID NO: 3)) was used as the hybridization probe for all blots. Hybridization was performed at 68°C for 1 hour in ExpressHyb (Clontech) with denatured probe at a concentration of 1×10^6 CPM/mL. Blots were then washed for 20 minutes in 2 X SSC/0.05% SDS at room temperature followed by high stringency washes at 50°C, 60°C, or 65°C in 0.1 X SSC/0.1% SDS for 20 minutes per wash and exposed to Kodak XAR film with an intensifying screen.

Please replace the paragraph at page 67, lines 3 through 7 with the following paragraph:

A16

Fusion proteins consisting of amino terminal regions of SExCkine fused to a C-terminal Histadine (His) were made in pEF-His or pEF1/V5-His A from Invitrogen (Carlsbad, CA) and fusion proteins consisting of N-terminal SExCkine regions fused to human alkaline phosphatase with a C-terminal His tag were made in the pDERF-SEAP vector (Yoshie, O., *et al.*, *J. Leukoc. Biol.*, 62(5):634-644 (1997)).

Please replace the paragraph at page 67, lines 8 through 16 with the following paragraph:

A17
The alkaline phosphatase fusion was produced by amplifying human SExCkine cDNA (SEQ ID NO:3) by PCR using a 5' synthetic oligonucleotide primer that contained a SalI site (5'cgcgctcgacagccgagatgggacgggacttg3', SEQ ID NO:12) and a 3' synthetic oligonucleotide primer that contained a XbaI site (5'ggtctagatgtcctggctgtgggacca3', SEQ ID NO:13). The 5' primer (SEQ ID NO:12) annealed to nucleotides 15-29 of SEQ ID NO:3 and encodes a protein beginning at the initiating Met (amino acid residue 1 of SEQ ID NO:4) and the 3' primer (SEQ ID NO:13) annealed to nucleotides 602-622 of SEQ ID NO:3. The PCR was run for 30 cycles (95°C (30 seconds), 55°C (30 seconds), 72°C (1 minute)).

Please replace the paragraph at page 67, line 25 through page 68, line 7 with the following paragraph:

A18
Additional constructs encoding fragments of the extracellular domain of SExCkine were made by PCR using a 5' primer (SEQ ID NO:14) and synthetic primer KHLL 3' (5' ggt cta gaa agt aaa tgc ttc tgg tgg gc 3', SEQ ID NO:16) or synthetic primer LMS 3' (5' cct cta gag ctc atc aat tcc tga acc c 3', SEQ ID NO:17) or synthetic primer 155 3' (5' ggt cta gac tgg gag ggt ggg gcg ctg ag 3', SEQ ID NO:18). Primer KHLL 3' annealed to nucleotides 345-364 of SEQ ID NO:3, and the product of the amplification reaction encoded residues 1 to 117 of SExCkine (SEQ ID NO:4). Primer LMS 3' annealed to nucleotides 280-300 of SEQ ID NO:3, and the product of the amplification reaction encoded residues 1 to 95 of SExCkine (SEQ ID NO:4). Primer 155 3' annealed to nucleotides 457-477 of SEQ ID NO:3, and the product of the amplification reaction encoded residues 1 to 155 of SExCkine (SEQ ID NO:4). Primers KHLL 3', LMS 3' and 155 3' each contained an XbaI restriction site.

Please replace the paragraph at page 68, line 19 through page 69, line 4 with the following paragraph:

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09/40063-082701
T04280-E900450

Thirty 10 cm plates (Beckton Dickinson) were seeded with 1×10^6 293T cells in DMEM +10% FCS. The next day the 293T cells were transfected by adding 10 μ g SExCkine/SEAP DNA to 790 μ L opti-MEM (800 μ L total) and mixing it with a solution of 60 μ L LipofectAMINE™ 2000 in 740 μ L opti-MEM (800 μ L total). The mixture was incubated at room temperature for 30 min, an additional 6.4 mL of opti-MEM was added to the mixture, and the mixture was added to the plates containing 293T cells. The plates were incubated at 37°C for 3 hours, then 8.0 mL DMEM+ 20% FCS was added. 24 hours later the transfection mixture was removed, the plates were washed with 1 X PBS, and 10 mL of serum free DMEM were added. The plates were then incubated for 3 days. The media (culture supernatant) was removed and filtered (500 mL filter bottle) to remove cellular debris. The harvested media was assayed for chemotactic activity using Bonzo/L1.2 cells essentially as described above. In addition, dilution curves were generated using supernatant diluted in media in a range of undiluted to 1:16 to assess general activity.

Please replace the paragraph at page 70, line 19 through page 71, line 6 with the following paragraph:

A20

A panel of antibodies which bind human Bonzo were produced by immunizing mice with transfected L1.2 cells that expressed high levels of Bonzo. The antibodies specifically bound to Bonzo expressed on the surface of Bonzo/L1.2 cells (Figures 8A-8D) but did not bind to transfected L1.2 cells which expressed CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, GPR5, V28, GPR9-6, Bob, LyGPR, AF, APJ or RDC (Figures 9A-9G). The antibodies, (e.g., mAbs 4A11, 7A2 and 7F3) inhibited the binding of SExCkine to Bonzo (Figure 10, Figure 31, Table 1). Staining studies revealed that Bonzo is expressed on small populations of CD4⁺ and CD8⁺ T cells as well as on CD16⁺/CD56⁺ NK cells. However, no expression of Bonzo was observed on CD19⁺/CD20⁺ B cells or on CD14⁺ monocytes (Figures 11A-11H). Multi-color staining studies were performed to analyze the co-expression of Bonzo and other cell surface proteins (Figures 12A-12D, 13A-13J, 14A-14H, 15A-15C, 16A-16D). These studies revealed that Bonzo is expressed predominantly on CD45RO^{hi} memory lymphocytes. Furthermore, Bonzo expression was detected on both skin